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Columbia Environmental Research Center

**LABORATORY EVALUATION OF THE
BEHAVIORAL AVOIDANCE-PREFERENCE
RESPONSE OF CHINOOK SALMON
(*Oncorhynchus tshawytscha*) TO
CHROMIUM IN THE HANFORD REACH OF
THE COLUMBIA RIVER, WASHINGTON,
USA.**

Prepared for the Hanford Natural Resource Trustee Council

**U.S. Department of the Interior
U.S. Geological Survey**

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UNITED STATES GEOLOGICAL SURVEY REPORT:

**LABORATORY EVALUATION OF THE BEHAVIORAL AVOIDANCE-
PREFERENCE RESPONSE OF CHINOOK SALMON (*Oncorhynchus tshawytscha*)
TO CHROMIUM IN THE HANFORD REACH OF THE COLUMBIA RIVER,
WASHINGTON, USA.**

PREPARED FOR:

The Hanford Natural Resource Trustee Council

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DISCLAIMER

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SUMMARY

The Hanford Nuclear Reservation in south central Washington was claimed by the federal government as a site for the production of plutonium. During the course of production and operation of the facilities at Hanford, radionuclides and chromium were discharged directly to the river and also contaminated the groundwater. Discharge and seepage of contaminated groundwater from the 100 Area into the Columbia River may be exposing salmon and other aquatic life to elevated levels of chromium, and the potential effects of these exposures remain a concern to area Natural Resource Trustees.

The Hanford Natural Resource Trustee Council in conjunction with the U.S. Fish and Wildlife Service and the U.S. Geological Survey designed a series of studies to assess the effects of chromium (Cr) on chinook salmon (*Oncorhynchus tshawytscha*) under exposure conditions similar to those that may exist in the Hanford Reach of the Columbia River. This study builds upon previous studies (Frag et al. 2000, Patton et al. 2000) and is a continuation of those efforts.

In this study the avoidance-preference responses of chinook salmon to aqueous chromium were evaluated using laboratory behavioral experiments. The parr life stage was used to facilitate laboratory experimentation. Two experiments were conducted. In the first, chinook salmon parr were individually placed in experimental chambers and presented with a choice between reconstituted Hanford experimental water (80 mg/L hardness as CaCO_3) and Hanford experimental water with the addition of chromium. Chromium concentrations ranged from 0 to 266 $\mu\text{g/L}$. These concentrations are within the range of concentrations that may be expected to occur in areas used by chinook salmon. Hexavalent chromium concentrations ranging from non-detectable to 632 $\mu\text{g/L}$ have been measured in pore water samples collected from the 100 Areas (Hope and Peterson 1996). The current ambient water quality criteria (AWQC) established for the protection of aquatic life (USEPA 1986) is 11 $\mu\text{g/L}$. Concentrations of chromium measured in the pore water exceeded the AWQC at 19 of 100 locations in the 100 D/DR Area (Hope and Peterson 1996a). Pore-water surveys conducted in the 100-H Area detected chromium above the AWQC at 3 of 31 sampling locations (Hope and Peterson 1996b).

In the second experiment chinook salmon parr, using the same apparatus and procedures, were presented with a choice between Hanford experimental water and a simulated Hanford groundwater (200 mg/L hardness as CaCO_3) with the addition of chromium. This second experiment attempted to simulate a scenario where chinook salmon encounter undiluted groundwater contaminated with chromium. Since chromium contamination in the Hanford Reach is associated with groundwater, it was thought that other water quality variables associated with upwelling groundwater might influence the response of chinook salmon to chromium. Many salmonids have exhibited a preference for habitats where significant upwelling of groundwater occurs (Geist 2000). Conversely, water quality

variables, such as increased hardness, often associated with groundwater have been documented to influence the ability to detect or respond to dissolved metals (Hartwell et al. 1987, Woodward et al 1995).

Results of avoidance-preference trials from Experiment I indicate that chinook salmon are capable of detecting and avoiding relatively low concentrations of dissolved chromium. The amount of time spent in chromium-treated water declined with increasing chromium concentrations. Concentrations $\geq 54 \mu\text{g/L}$ caused a statistically significant avoidance response in chinook salmon parr when compared to fish under control conditions. The response of fish presented with the next lowest concentration tested ($27 \mu\text{g/L}$) was not significantly different from fish under control conditions. Under these test conditions the avoidance threshold for chromium to chinook salmon is assumed to be between 27 and $54 \mu\text{g/L}$. This data is consistent with observations made by Anestis and Neufeld (1986). In avoidance-preference experiments conducted using sub-adult rainbow trout (*Oncorhynchus mykiss*) they calculated an avoidance threshold for chromium of $28 \mu\text{g/L}$. Similarly, Hartwell et al. (1989) reported an avoidance threshold of $73 \mu\text{g/L}$ chromium for golden shiners (*Notemigonus crysoleucas*). Avoidance responses in the field can have long-term and far-reaching effects on sensitive anadromous fish populations. Avoidance of contaminated habitat may reduce available habitat, impact reproduction and juvenile production, and impair imprinting and homing behavior. The extent to which chromium avoidance may impact salmon in the field should be carefully evaluated based upon the extent of current and past contamination, the timing of exposure and the life stage exposed. For example, our experiments examined the response of parr salmon to chromium exposure. Due to practical considerations the behavioral responses to chromium exposure of adult or early life stage salmon were not directly evaluated in the laboratory. These life stages may be more or less sensitive than parr salmon. Caution must also be exercised when extrapolating laboratory data to field conditions.

Results from Experiment II were more complex and difficult to interpret. In contrast to Experiment I, data show that chinook salmon parr failed to avoid aqueous chromium concentrations ranging from 11 to $266 \mu\text{g Cr/L}$ when chromium was presented in water of increased hardness (simulated Hanford groundwater). Under these conditions, all treatments responded similarly. However, closer examination of the data reveals that the responses of individual fish were more variable than in Experiment I. This increased variation in the avoidance-preference response occurred among fish from all treatments, including fish presented with uncontaminated, simulated groundwater. While salmon parr did not exhibit a marked preference for simulated groundwater alone, it is clear that fish presented with chromium in simulated groundwater did not respond in the same manner as the fish in experiment I. Various factors may have contributed to this difference in behavioral responses between the experiments including, complexation of chromium, the acclimation

history of the test organisms, competing motivational variables, and the alteration of the perception of chromium by salmon due to the water quality changes accompanying simulated groundwater. However, one potential implication of these findings is that salmon may not be capable of discriminating between contaminated and uncontaminated habitat when chromium is presented in undiluted groundwater. Under this scenario life-stages of salmon utilizing this habitat may not be able to behaviorally mitigate their exposure. Information on the extent of contamination, discharge rates, dilution, and life stage present must all be evaluated in order to assess the potential for effects in the field.

The avoidance-preference response is the primary response of organisms to an environmental contaminant. Avoidance of environmental contaminants is an adapted behavior that often reduces exposure to contaminants through behavior that may limit contact with, or residence in, unfavorable or contaminated habitat. Significant behavioral avoidance of contaminated areas may result in the substantial loss of important habitat. On the other hand, failure to avoid contaminated areas or preference for contaminated areas may result in increased exposure to hazardous substances leading to physiological impairment or death. Chinook salmon are capable of detecting and avoiding concentrations of chromium that may be expected to occur in the Hanford Reach. However, this avoidance response to chromium can be altered by other biological and environmental factors (such as hardness or turbidity). Concentrations avoided by chinook salmon are similar to concentrations shown in laboratory studies to result in tissue accumulation in early life stage salmon (Patton et al. 2000) and are within the range of concentrations known to result in physiological impairment in salmon parr (Frag et al. 2000).

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INTRODUCTION

The Hanford Nuclear Reservation in south central Washington is a 900 square km area claimed by the federal government in 1943 as a site for the production of plutonium (Figure 1)(Geist 1995). The location was ideal because it was remote, sparsely populated, and most importantly, had a readily available supply of cold water from the Columbia River. Because of national security concerns, public access and river development projects were restricted until 1971 (Dauble and Watson, 1997). Extensive dam building and development occurred throughout the Columbia River Basin from 1943 to 1971 and led to severely reduced populations of chinook salmon (*Oncorhynchus tshawytscha*). The 90 km section within the Hanford Reservation was not developed, and today, the Hanford Reach remains a free flowing stretch of the Columbia River and is the only remaining area where significant mainstem salmon spawning occurs in the Columbia River (Dauble and Watson 1990). Though upstream dams regulate flows within the Hanford Reach, it is the last unimpounded stretch of the mainstem Columbia River. As a result the use of the Hanford Reach for fall chinook salmon spawning and rearing has dramatically increased since 1960 (Becker 1985, Dauble and Watson 1990). The 10-year average adult escapement increased from 27,660 (1964-1973) to 54,661 (1983-1992). This increase is pronounced when compared with the rest of the mid and upper Columbia River where chinook salmon runs have declined during the same time period.

During operation of the Hanford facilities large quantities of Columbia River water were used to cool nuclear reactors, and cooling water was treated with sodium dichromate to prevent corrosion and mineral collection within the pipes (Peterson et al. 1996). During operations, cooling water with associated radionuclides and chromium was discharged directly to the river and also entered groundwater through leakage of pipes and seepage from retention areas. Today, groundwater at the Hanford site continues to be contaminated with chemical and radiological constituents (Geist et al. 1994). The hydraulic head of the groundwater aquifers in the 100 Area (National Priority List Site) are higher in elevation than that of the Columbia River, which results in discharge from the aquifer into the Columbia River through shoreline springs and seeps (Figure 2). The groundwater is hydraulically connected to the river with peak aquifer discharges occurring during low river flows (fall and winter) and minimum aquifer discharges occurring during high river flows (spring and summer) (Geist et al. 1994). These periods of peak aquifer discharge coincide with the spawning and early developmental periods of fall chinook salmon.

Redd counts conducted over the last several decades indicate that the majority of chinook salmon spawning occurs at several discrete areas within the Hanford Reach (Dauble and Watson 1997). Within these important spawning areas such as Locke Island, which is adjacent to the 100H Area, as little as 9 to 22 % of the riverbed may be suitable as spawning

habitat for fall chinook salmon (Geist et al. 2000). Important spawning areas are in proximity to sites in the 100 Area where contaminated groundwater is entering the river. This indicates the likelihood that some spawning salmon and their resultant developing eggs and larvae may be exposed to elevated levels of chromium. Trustees responsible for the aquatic resources in this reach of the river need to be able to adequately assess the potential for contaminated groundwater to impact chinook salmon.

Adult chinook salmon spawn in variable water depths, water velocities, and substrate types (Swan et al. 1988). Spawning in the Hanford Reach begins in mid-October, peaks in mid-November, and ends in late November (Dauble and Watson 1997). Egg and fry development within the redds takes place from mid-October to May during low river flows that result in peak aquifer discharges. Based on the mid-November peak redd abundance and ambient temperatures, eggs would become eyed in early December, hatch in late December, and alevins would emerge from the redds in late February. Upon emergence, fry move out of the main river channel into shallow, slow moving, near shore and backwater habitat (Dauble and Watson 1990, Dauble et al. 1989). Juveniles remain in the Hanford Reach from February to mid-July feeding on macroinvertebrates (Becker 1973). Outmigration begins in May and is usually completed by July at 5-7 months of age, 60-70mm in length, and 3-4g in weight (Olson and Foster 1956).

Chromium is a contaminant of major concern associated with the 100 Area groundwater and seeps. Concentrations of chromium measured in the pore water exceeded the U.S. EPA (1986) chronic ambient water quality criteria for the protection of aquatic life (AWQC) at 19 of 100 locations in the 100 D/DR Area (Hope and Peterson 1996a). Pore water surveys conducted in the 100-H Area detected chromium above the AWQC at 3 of 31 sampling locations (Hope and Peterson 1996b). The highest concentration of chromium reported was 632 µg/L. Areas of habitat suitable for chinook salmon spawning and spawning redds have been documented in or near areas with contaminated upwelling groundwater at both the 100D/DR and 100-H areas (Hope and Peterson 1996a, 1996b). While the actual amount of physical habitat impacted by chromium contamination has not been quantified, pore water data suggest that areas with the highest contamination (>50 µg/L) are relatively localized within the reaches that have been sampled to date and many are associated with current or planned groundwater remediation activities. The Department of Energy is currently employing pump and treat technology as well as *in-situ* treatment using a permeable reactive barrier to reduce the amounts of hexavalent chromium released into the Hanford Reach. However, the critical nature of the Hanford Reach as spawning habitat for the chinook salmon, makes it essential to determine the potential for chromium in the groundwater to adversely impact chinook salmon (Geist 1997). Until the present there has been little data (Olson and Foster 1956, Buhl and Hamilton 1991) that described the effects of chromium on salmon. Recently completed studies in this project have focused on the

potential impacts of chromium exposure on fertilization, early life-stage effects and physiological impairment (Farag et. al 2000, Patton et. al 2000).

The goal of the experiments described in this report was to determine whether chinook salmon exhibit an avoidance response under laboratory conditions to chromium concentrations present in the Hanford Reach of the Columbia River. Fish may avoid concentrations of contaminants well below those levels that may cause mortality or reductions in growth (Little et al. 1985). Avoidance of elevated concentrations of environmental contaminants can alter the distribution of fish in the field and affect habitat use, intra-specific competition, growth and mortality (Woodward et al. 1995, DeLonay et al. 1996, Lipton et al. 1996, Hansen et al. 1999). Chromium avoidance thresholds reported in the literature for other species are within the range of concentrations expected to occur in the Hanford Reach of the Columbia River. Anestis and Neufeld (1986) reported an avoidance threshold of 28 µg/L for rainbow trout (*Onchorhynchus mykiss*) exposed to aqueous chromium. An avoidance threshold level of 73 µg/L chromium has been reported for golden shiners (*Notemigonus crysoleucas*) (Hartwell et al. 1989). Documentation of laboratory avoidance may indicate the potential for chromium contamination to adversely impact habitat quality and availability for early life-stage chinook salmon in the Hanford Reach of the Columbia River.

The Hanford Natural Resource Trustee Council in conjunction with the U.S. Fish and Wildlife Service and the U.S. Geological Survey designed this study to assess the behavioral avoidance-preference response of chinook salmon to chromium under exposure conditions that may exist in the Hanford Reach of the Columbia River. To achieve this objective two experiments were conducted.

Experiment I. Determine the avoidance response of chinook salmon to aqueous chromium concentrations under conditions representative of the Hanford Reach of the Columbia River (80 mg/L hardness as CaCO₃).

Experiment II. Determine the avoidance response of chinook salmon to aqueous chromium concentrations in simulated Hanford groundwater (200 mg/L hardness as CaCO₃).

METHODS

The goal of these experiments was to assess the potential for chromium to influence chinook salmon distribution and habitat availability in the Hanford Reach of the Columbia River. The first step in this assessment was to determine whether chinook salmon exhibit an avoidance or preference response to chromium under controlled laboratory conditions. Laboratory tests measure the response of salmon presented with a choice between a control condition and chromium-contaminated water (the test condition or treatment). Precisely controlled conditions are necessary to ascribe the observed behavioral response to the presented stimulus. Water quality simulating conditions that may occur in the Columbia River were used to control variables such as hardness and pH that are known to affect the speciation, complexation, biological availability and toxicity of metals, such as chromium. Although test conditions attempted to simulate many of the conditions experienced by fish in the field, the focus of laboratory tests was to control the nature of the stimulus (aqueous chromium) and the conditions under which it was presented to the organism. Experiments were conducted with the parr life-stage to facilitate laboratory testing and to provide data upon which inferences about other life stages may be made.

Two avoidance experiments were conducted. The first, Experiment I, determined the behavioral response of chinook salmon to chromium concentrations ranging from 0 to 266 $\mu\text{g/L}$ in reconstituted, Hanford experimental water (80 mg/L hardness as CaCO_3 , $10 \pm 2^\circ\text{C}$). Chromium concentrations were selected based on the chronic EPA ambient water quality criteria (EPA 1986) of 11 $\mu\text{g/L}$, concentrations that were expected to elicit an avoidance response based on a survey of the literature (Anestis and Neufeld 1986), and the range of concentrations expected to occur in the Hanford Reach of the Columbia River (Hope and Peterson 1996). Selected exposure concentrations and the test matrix for Experiment I are illustrated in Table 1. In each treatment combination an individual fish is presented with a choice between the control side of an experimental chamber (uncontaminated Hanford experimental water) and the treated side of the chamber (Hanford experimental water contaminated with 0 to 266 $\mu\text{g/L}$ chromium).

The second experiment, Experiment II, was designed to examine whether the water quality characteristics associated with an undiluted groundwater source would alter the response of chinook salmon to chromium. Chromium is associated with seeps and areas of upwelling, contaminated groundwater that exist along the river's edge, and in the riverbed. Water quality characteristics associated with upwelling groundwater (i.e., increased hardness) may alter the avoidance response by either changing the perception or toxicity of the stimulus (chromium), or by presenting water quality conditions (hardness, alkalinity, pH, etc.), which may be preferred over the control condition. This second experiment will evaluate the response of chinook salmon to a simulated groundwater (200 mg/L hardness as CaCO_3) with the addition of aqueous chromium concentrations ranging from 0 to 266 $\mu\text{g/L}$.

The selected exposure combinations and the test matrix for Experiment II are illustrated in Table 1. In each treatment combination an individual fish is presented with a choice between the control side of an experimental chamber (uncontaminated Hanford experimental water) and the treated side of the chamber (simulated Hanford groundwater contaminated with 0 to 266 µg/L chromium).

SELECTION OF TEST ORGANISMS:

Eyed embryos of fall-run chinook salmon were obtained from the McNenny State Fish Hatchery, Spearfish, South Dakota. Eggs were maintained in a Heath^R incubator at a temperature of 10 ± 2 °C and hardness of approximately 300 mg/L as CaCO₃ through hatch. At hatch, the fish were moved to flow-through laboratory raceways until of sufficient size for behavioral testing (0.25 - 2.0 g). Juvenile chinook salmon were acclimated to, and maintained in Hanford experimental water (80 mg/L hardness) at the test temperature (10 ± 2 °C) for a minimum of three weeks prior to the start of the experiments. The fish were fed at least a 5% wet weight ration of a commercial biodiet daily. The daily food ration was split between two feedings. Fish were not fed for 24 hours prior to testing. Fish used in the behavioral tests ranged in size from 4.8 to 6.0 cm (TL) and weighed between 0.9 and 1.8 g.

Eyed embryos, larvae, and juveniles were handled so as to minimize stress in accordance with the CERC-Columbia Animal Welfare Plan and the Region 6 U.S. Fish and Wildlife Service, Fish Health Policy. Experiments required the use of naïve fish or fish with no prior experience with the testing apparatus. Therefore an individual fish was tested only once.

Salmon from the McNenny Hatchery were selected for use in these studies by the Trustees based upon consideration of the following:

- 1.) Chinook salmon from the Hanford Reach may be exposed to significant concentrations of chromium, and several other contaminants during development and residence in the Reach (Geist et al. 1994). The potential effects of these contaminants on chinook salmon are unknown. A history of pre-exposure to environmental contaminants of test organisms could potentially bias or confound test results (ASTM 2000a). Selection of a fall-run chinook salmon stock from the McNenny Hatchery eliminated the potential confounding effects due to possible pre-exposure.
- 2.) While evolutionary adaptation has resulted in stocks of salmon that exhibit distinct differences in life history and reproductive site fidelity, these ecological adaptations would not likely result in significant differences in the tolerance or sensitivity to anthropogenic contaminants of relatively recent origin, such as hexavalent chromium (Mayer and Ellersieck 1986). Evidence of adaptation to environmental

concentrations of chromium may in, and of itself, constitute a biological effect of exposure and result in altered viability of natural populations.

- 3.) Data collected from laboratory avoidance-preference studies will be directly comparable to earlier studies conducted at the Columbia Environmental Research Center and the Jackson Field Research Station.
- 4.) Adult brood fish from the McNenny Fish Hatchery are examined and tested for disease and parasite infection during spawning, and the eggs certified disease free prior to testing or shipment to Columbia, Missouri and Jackson, Wyoming. The disease free status is essential in assuring that toxicity testing is performed on healthy test organisms, increases reliability of results, and is a recommended standard procedure (ASTM 2000b).
- 5.) Chinook salmon from the McNenny Hatchery have been used as a source for test organisms in past Natural Resource Damage Assessments (Blackbird Mine Site, Idaho; Marr et al. 1995). Use of a single source for test organisms provides a consistent baseline of data that can be applied by Trustees to other contaminant releases and contaminated sites.

EXPERIMENTAL CONDITIONS:

Hanford experimental water was reconstituted in the laboratory to simulate the Columbia River surface and pore water quality in the Hanford Reach and conditions known to be associated with the location of spawning redds (Hope and Peterson 1996, Geist 1997). Hanford experimental water was adjusted to a hardness of 80 mg/L as CaCO₃; pH, alkalinity, and conductivity were maintained in a range consistent with Columbia River conditions. Simulated Hanford groundwater was reconstituted in the laboratory and adjusted to a hardness of 200 mg/L as CaCO₃; pH, alkalinity, and conductivity were maintained in a range consistent with groundwater conditions near the Columbia River.

Reconstituted waters used in this study were prepared by blending laboratory well water with deionized water produced by reverse osmosis. This eliminated the use of surface water and the potential for fish pathogens or other constituents and contaminants to be introduced into the experiment and influence test results. Reconstituted waters were produced in 5,600L batches and analyzed to insure quality was within 5% of the experimental design in terms of hardness, alkalinity, conductivity, and pH. Unless otherwise indicated, reconstituted waters were used. Photoperiod was adjusted to simulate time of year of the exposure. Behavioral experiments were conducted at 10 ± 2 °C and matched seasonal conditions expected to occur in March through July (Wiggins et al. 1997).

EXPERIMENTAL APPARATUS:

Avoidance-preference experiments were conducted using a counter-current apparatus similar to that used by Sprague (1968) and procedures developed by CERC for rapid determination of behavioral avoidance-preference responses in Natural Resource Damage Assessment injury determinations (DeLonay et al. 1996, Lipton et al. 1996). This apparatus uses an experimental chamber, which produces a steep, central gradient between a control, and a test treatment. The experimental chamber consists of a Plexiglas cylinder (11 cm diameter x 92 cm) with six centrally located drain holes (Figures 3 and 4). Openings have been cut in the surface of the cylinder to allow the addition and removal of fish and to allow the placement of screens at each end. The screens are located 11 cm from each end of the chamber, creating a 70 cm observation area into which a single fish is introduced. A control (water without chromium) and a treatment solution (water with chromium) flow in from opposite ends and exit from the six adjustable drains at the center of the chamber.

During operation of the apparatus, water of the appropriate quality is pumped from temperature-controlled reservoirs into both ends of the chamber (Figure 5). The flow rate is controlled by adjustable valves and monitored by high-accuracy infrared flow sensors. Chromium is introduced into the treatment side of the test chamber using a high-accuracy Masterflex® digital metering pump. Switching solenoids are used to randomize and alternate the side of the experimental chamber that receives the chromium treatment and/or the simulated Hanford groundwater. For this study, three of these experimental systems were constructed and operated simultaneously (Figure 6).

Prior to the start of the experiments each experimental chamber was calibrated, the operation of all components verified and the steepness of the gradient documented using fluorescein dye. Pre-experiment trials were conducted with chinook salmon to determine the appropriate acclimation period. Information from these trials indicated that 40 minutes was sufficient for juvenile chinook salmon to acclimate to the experimental conditions and to begin freely moving about the chamber. Acclimation times are often species and life-stage dependent and have usually ranged from 20 to 40 minutes for other salmonids in this apparatus.

AVOIDANCE-PREFERENCE EXPERIMENTS:

In each experiment a series of trials were conducted using all three chambers concurrently. Within a trial each of the chambers delivered the same treatment combination (Table 1). Chambers within a trial were not considered to be independent; therefore observational data from the three chambers within a trial were pooled. Each treatment combination always contained a control or reference condition (0 µg/L Cr) on one side of the chamber and a treatment condition on the opposite side of the chamber. All six of the treatment combinations within a replicate were tested within the same day. The treatment condition delivered to each test chamber, and the end of the chamber receiving the treatment

condition was randomized and alternated between trials. The series of treatment combinations was replicated eight times (eight replicates of three fish each) for each experiment. Therefore each experiment required the observation of 288 individual fish (3 chambers x 8 replicates x 6 treatment combinations). The control treatment combination for the experiment was defined as the test in which experimental water without contaminants (0 µg/L Cr) flows into both ends of the chamber.

Behavioral trials consisted of a 40-minute acclimation period followed by a 40-minute test period. During the acclimation period individual fish were randomly placed into one end of each of three experimental chambers as uncontaminated water flowed into both ends of the chamber. After 40 minutes the test was initiated by turning on a high-accuracy Masterflex® digital metering pump to introduce chromium into one end of the test chamber. The test period continued for 40 minutes. The behavioral response to the contaminant gradient was recorded on videotape for later analysis.

Based upon calibration information, the first ten minutes of the test period were required to ensure that the nominal test concentration was reached and a stable gradient was established within the chamber. A second 10-minute period was allowed for the fish to detect and respond to the contaminant gradient. The final 20-minute interval was selected as the observation period. Behavioral response data was recorded from this period as the proportion of time spent in the test solution versus the time spent in the control solution. The frequency of gradient crossing by fish into the treatment side of the chamber during the observation period (number of trips) and the residence time per gradient crossing into the treatment condition (trip time) during the observation period were also recorded. Frequency and residence time metrics were used to evaluate the severity and mechanism of the behavioral response. These measures were also used to examine the consistency of the response between experiments and may be used to compare data among species.

All three chambers were enclosed in a structure to shield against external movement and sound. Water quality characteristics (pH, alkalinity, hardness, and conductivity) of the Hanford experimental water and the simulated Hanford groundwater were sampled daily. Tests were discarded if there was a disturbance to the avoidance apparatus; inconsistent water chemistry, temperature, or quality; or disease, or abnormal behavior. Tests were also discarded if test organisms did not cross the gradient a minimum of three times during the test period. Responses from only 3 fish (each from different replicates and concentrations) out of a total of 288 tested were discarded based on these criteria.

ANALYSIS OF CHROMIUM:

Aqueous samples for the determination of chromium concentrations were taken from one of the three chambers for each concentration, within each replicate. One hundred mL samples were filtered using a Nalgene® 300 filter holder. Each filtered sample was transferred to a pre-cleaned, 125 mL I-Chem® polyethylene bottle, acidified to 1% HNO₃,

and analyzed with ICP-MS. All chromium was assumed to be in the hexavalent state due to the short duration of the exposure. Therefore, speciation of samples was not determined.

STATISTICAL INTERPRETATION OF RESULTS:

Statistical analyses were performed using SAS system software, version 8.0 (SAS Institute Inc., Cary, North Carolina). Analyses of Variance followed by Tukey means comparisons were performed on all data that met the assumptions of homogeneity and normality. The number of replicates for each experiment was eight. Statistical significance was assigned at $P \leq 0.05$.

RESULTS

EXPERIMENTAL CONDITIONS:

Test conditions during these experiments were within specifications set out in the study protocol. Water temperature in the experimental chambers ranged between 9.5 and 10.8°C. Measured temperatures did not change during any trial by more than 0.4 °C. Differences in temperature between ends of the same chamber never varied by more than 0.2 °C. Ranges of water quality characteristics for Hanford experimental water used in Experiment I and II were as follows: alkalinity ranged from 70.5 to 74 mg/L as CaCO₃; conductivity from 185 to 192 µS/cm; hardness from 78.5 to 80.5 mg/L as CaCO₃; and pH from 8.1 to 8.3. Ranges of water quality characteristics for simulated Hanford groundwater used in Experiment II were as follows: alkalinity ranged from 175 to 182 mg/L as CaCO₃; conductivity from 452 to 460 µS/cm; hardness from 199.5 to 201 mg/L as CaCO₃; and pH from 8.1 to 8.3.

Concentrations of total chromium measured in the exposure chambers (Table 2 and Table 3) agreed well with the nominal concentrations for both Experiments I and II. Concentrations of total chromium were within $\pm 10\%$ of the nominal concentrations stated in the quality assurance guidance plan. Percent recoveries of reference solutions and spikes were $\geq 99\%$. Quality control was within acceptable limits specified by CERC. Therefore, from this point forward discussion of results will refer to nominal chromium concentrations.

Weight and length of Chinook salmon tested in Experiments I and II were similar among all treatments (Table 4 and Table 5). Mean weights ranged from 1.2 to 1.4 g and mean lengths ranged from 5.4 to 5.7 cm (TL).

EXPERIMENT I:

In Experiment I salmon parr were presented with a choice between a control condition (experimental Hanford water at 80 mg/L hardness as CaCO₃) and a test condition (experimental Hanford water at 80 mg/L as CaCO₃) to which chromium had been added. The behavioral response of control fish indicated that the test apparatus was calibrated properly and that no extraneous variables were influencing the distribution of fish within the test chambers. Fish presented with control conditions on both sides of the chamber (no chromium) spent nearly identical amounts of time on both sides of the chamber (Figure 7, Table 6). Under ideal control conditions the distribution of fish should approach an even 50% distribution between time spent on the control side and time spent on the treatment side of the apparatus.

Behavioral data from Experiment I clearly indicate that chinook salmon parr can detect and avoid very low concentrations of aqueous chromium (Figures 7, Table 6). Fish presented with a choice between experimental Hanford water without chromium and experimental Hanford water with chromium spent less time in the treatment side of the

chamber and more time in the control, or uncontaminated side of the chamber. This response became more pronounced with increasing chromium concentration. Salmon presented with concentrations ≥ 54 $\mu\text{g/L}$ exhibited a statistically significant avoidance response as compared to the response of fish in the absence of chromium. The highest concentration of chromium not producing a statistical avoidance response was 27 $\mu\text{g/L}$.

There was no difference among treatments in the frequency of gradient crossings (trips into the contaminated side of the chamber) in Experiment I (Figure 8, Table 6). However, the residence time for each trip into the chromium-treated side of the chamber declined with increasing concentration (Figure 9, Table 6). In summary, although salmon parr crossed into the chromium-treated side of the chamber the same number of times, the mean time of each excursion was significantly reduced with successively greater chromium concentrations. Fish from treatments with concentrations of chromium ≥ 54 $\mu\text{g/L}$ exhibited statistically reduced residence times per trip into the test side of the chamber when compared with fish in chambers without chromium.

EXPERIMENT II:

In Experiment II salmon parr were presented with a choice between a control condition (experimental Hanford water at 80 mg/L hardness as CaCO_3) and a test condition with water of a higher hardness (simulated Hanford groundwater at 200 mg/L as CaCO_3) to which chromium had been added. The behavioral response of salmon presented with the treatment combination of experimental water versus simulated groundwater without chromium indicated that salmon parr did not prefer or avoid either water quality (Figure 10, Table 7). Fish presented with both water qualities in the absence of chromium spent nearly identical amounts of time on both sides of the chamber. However, closer examination of the data reveals that the responses of individual fish were more variable than in Experiment I. This increased variation in the avoidance-preference response occurred among fish from all treatments, including fish presented with uncontaminated, simulated groundwater.

In contrast to Experiment I, data show that chinook salmon parr failed to avoid aqueous chromium concentration ranging from 11 to 266 $\mu\text{g Cr/L}$ when chromium was presented in water of increased hardness (simulated Hanford groundwater). Under these conditions, all treatments responded similarly. While salmon parr did not exhibit a marked aversion or preference for simulated groundwater alone, it is clear that fish presented with chromium in simulated groundwater did not respond in the same manner as the fish in experiment I (Figure 10, Table 7).

There was no difference among treatments in the frequency of gradient crossings (trips into the contaminated side of the chamber) in Experiment II (Figure 11, Table 7). In addition mean residence times for each trip into the chromium-treated side of the chamber were similar among all treatments (Figure 12, Table 7).

DISCUSSION

Many compounds, including metals, pesticides, chlorinated compounds, industrial chemicals, and complex effluents, are known to induce avoidance responses in fish (Atchison et al. 1987, Beitinger 1990). The ability to detect and avoid a contaminant provides a means of mitigating potentially deleterious exposures. However, avoidance may result in displacement of fish from preferred habitats to areas that are less optimal for survival in terms of shelter, food, reproduction or protection from predators (Atchison et al. 1987). In more extreme cases, avoidance of contaminants by aquatic organisms may result in the effective loss of habitable resources, the interruption of essential migratory behaviors and the loss of viable populations in the field (Sprague et al 1965; Saunders and Sprague 1967). Localized declines in fish populations and the loss of habitable resources due to the behavioral avoidance of environmental contaminants may alter aquatic ecosystems and cause significant biological and economic injury to natural resources. Avoidance responses, therefore, are an important measure of sublethal effect resulting from exposure to hazardous substances. These responses are particularly important because they can occur at toxicant concentrations substantially lower than lethal thresholds (Little et al. 1985; Little et al. 1993). Therefore, effective site remediation or restoration must consider behavioral avoidance when setting action limits and clean-up criteria.

We conducted laboratory experiments to examine the potential for chromium to influence chinook salmon distribution and habitat availability in the Hanford Reach of the Columbia River. Experiments were conducted to measure the response of salmon presented with a choice between a control condition and a treatment condition consisting of chromium-contaminated water. Water quality simulating the conditions occurring in the Columbia River were used to control variables such as hardness and pH that are known to affect the speciation, complexation, biological availability and toxicity of metals, such as chromium.

Data from Experiment I clearly indicates that under the set of experimental conditions tested chinook salmon are able to detect and avoid relatively low concentrations of chromium. Salmon parr avoided concentrations of chromium $\geq 54 \mu\text{g/L}$. The highest concentration not producing a statistically significant avoidance response when compared to the response of fish under control conditions was $27 \mu\text{g/L}$. These data suggest that the avoidance threshold of chinook salmon for chromium under these conditions lies between 27 and $54 \mu\text{g/L}$. These data are consistent with avoidance thresholds reported in the literature for other species (Table 8). Anestis and Neufeld (1986) reported a calculated avoidance threshold of $28 \mu\text{g/L}$ for rainbow trout exposed to aqueous chromium. An avoidance threshold level of $73 \mu\text{g/L}$ chromium has been reported for golden shiners (Hartwell et al. 1989).

Chromium avoidance occurred far below concentrations that have been reported to be acutely lethal for salmonids in general, and chinook salmon in particular. Benoit (1976)

reported 96-hr LC₅₀ values of 59 mg/L for brook trout (*Salvelinus fontinalis*) and 69 mg/L for rainbow trout. Buhl and Hamilton (1991) reported 96-hr LC₅₀ values ranging 71 mg/L to >600 mg/L for three salmonids including, Arctic grayling (*Thymallus arcticus*), coho salmon (*Oncorhynchus kisutch*) and rainbow trout. Hamilton and Buhl (1990) reported 96-hr LC₅₀ values for chinook salmon fry ranging from 111 to 144 mg/L. Chromium concentrations avoided by chinook salmon are similar to concentrations shown in chronic studies to result in significant tissue accumulation (Patton et al. 2000), reduced growth and increased mortality (Olson and Foster 1956) among early life stage salmon, and within the range of concentrations known to result in physiological impairment in salmon parr (Farg et al. 2000).

Although the results of Experiment II indicate that the salmon parr did not show a significant aversion or preference for simulated Hanford groundwater over experimental Hanford water, it is clear that the presentation of chromium in a water quality different from the acclimation and control conditions fundamentally influenced the avoidance response. This alteration of an avoidance response with changes in water chemistry has been observed in tests with other species and other metals (Hartwell et al. 1987, Woodward et al. 1995). The toxicity of many metals, such as copper and zinc, is modified by increased cation concentrations (calcium or magnesium) that accompany increased water hardness (Black et al. 1973). Reduced avoidance responses to metals have also been reported waters of increased hardness (Hartwell et al. 1987, Woodward et al. 1995). Evidence of the causative mechanism of action for these metals implicate the complexation of metals, which may alter their toxicity or the ability of fish to perceive them; reduced permeability of epithelial tissue to metals in the presence of high cation concentrations; and a reduction in olfactory tissue damage in the presence of increased cation concentrations (Hansen et al. 1999a, 1999b). From our experiments it is difficult to discern whether the cause of the altered response was due only to the influence of hardness on chromium, or whether other factors may have played a role as well. The acclimation history of the test organisms and the influence of competing motivational variables may have influenced the response to some degree. This is evidenced by the increased variability in responses among fish in experiment II. One potential implication of the failure of chinook salmon to avoid concentrations of chromium $\leq 266 \mu\text{g/L}$ is that salmon may be unable to behaviorally mitigate their risk of exposure under some exposure scenarios. For example, spawning salmon may not be able to differentiate chromium-contaminated spawning sites from uncontaminated sites if upwelling water consists primarily of groundwater. Salmonids selecting spawning sites based upon groundwater flow would effectively increase the potential exposure for their developing progeny.

Avoidance of metal-contaminated habitat has been documented to influence the distribution of fish in the field. Sprague et al. (1965) and Saunders and Sprague (1967) reported that copper and zinc contamination from mining activity in the drainage of a New Brunswick

stream reduced upstream migration of returning adult Atlantic salmon. Geckler et al. (1976) observed avoidance behavior in a stream, Shayler Run, which was intentionally dosed with copper. Resident fish populations mediated exposure by actively seeking out areas of low copper concentration. Hartwell et al. (1987) documented avoidance of a mixture of copper, chromium, arsenic, and selenium by fathead minnows (*Pimephales promelas*) in the laboratory and natural streams. Woodward et al. (1995) postulated that avoidance of low concentrations of metals associated with mining activities was in part responsible for the distribution and decline of salmonids in the Clark Fork River.

Significant avoidance of chromium occurs in the laboratory within the range of concentrations expected to occur in the Hanford Reach of the Columbia River. Although chromium is diluted rapidly as contaminated groundwater migrates into the river channel, concentrations of hexavalent chromium in the groundwater upwellings of the Hanford 100 Areas have been documented to range from non-detectable to 632 µg/L in pore water from substrate at the bottom of the Columbia River (Hope and Peterson 1996a, 1996b). While the actual amount of physical habitat impacted by chromium contamination has not been quantified, pore water data suggest that areas with the highest contamination (>50 µg/L) are relatively localized within the reaches that have been sampled to date. Many of these areas are associated with current or planned groundwater remediation activities. However chinook salmon spawning data from the Hanford Reach suggest that relatively small areas of habitat are essential for salmon production. Giest et al. (2000) indicated that as little as 9 to 22% of the riverbed in areas of high reproductive activity may be suitable for spawning. Salmonids have been shown to be highly selective in their choice of spawning habitat. Some species are highly selective for areas of upwelling groundwater or river water. Giest (2000) reports that chinook salmon in the Hanford Reach may prefer spawning areas that are significantly influenced by hyporheic discharge. This hyporheic discharge was heavily influenced by river water that entered the substrate at locations upstream of the spawning locations. The permeability of river substrates strongly influences where these areas of discharge occur and the rapidity and extent to which groundwater mixes with hyporheic and surface water flows (Hope and Peterson 1996a). Pore water sampling locations where an impermeable, near-surface hardpan clay layer was present appeared to have higher chromium concentrations than areas with unconsolidated river substrates. Substrate permeability also has a significant positive influence on the suitability of habitat for spawning salmon. Available spawning data from the Hanford Reach and studies by Geist (1997) and others should be examined to evaluate the selection criteria used by spawning salmon and other species to locate reproductive habitat to determine whether some species or populations are at a greater risk of exposure than others.

In this study experiment I was designed to examine the response of chinook salmon to dissolved chromium in water quality that reflected conditions likely to occur in the Hanford reach as groundwater is rapidly diluted by river water. Experiment II was designed to

simulate a scenario where chinook salmon encounter undiluted chromium-contaminated groundwater. These exposures may occur as adult salmon actively seek potential spawning redds or as larvae developing in the interstitial spaces of the redd experience increased groundwater flow as a result of changes in river discharge or hydropower peaking operations. Significant avoidance of chromium in the field could result in reduced availability of spawning habitat or reproductive success as adults spawn in higher densities in the remaining suitable spawning sites. Rejection of spawning habitat due to existing or historical contamination may have long-term consequences for populations of anadromous fishes with high natal site fidelity and may in part explain why populations of anadromous salmonids are difficult to reintroduce into areas where they have been extirpated. Altered growth, development or out-migration behavior as a result of contaminant avoidance may influence year-class strength and production. Discharge of contaminated groundwater through redds where salmon larvae are developing may potentially elicit an avoidance response that may alter the timing and maturity of juvenile salmon at emergence. In addition sensitive stages of imprinting in early life-stage salmonids may be disrupted by the olfactory epithelial damage caused by exposure to metals, such as chromium. While each of these potential impacts may have far-reaching and serious consequences, most are undocumented and remain to be examined thoroughly for chinook salmon or any other species, and chromium specifically.

Application of the results of this study to determine the potential for chromium to adversely affect the behavior and distribution of chinook salmon, and other species, in the field should include life history and behavioral information for the life-stages present, the extent of their proximity to chromium sources, and a realistic approximation of the prevailing environmental conditions within that habitat. There is a need for an integrated assessment to evaluate the amount and biological value of habitat influenced by chromium contamination; the level of contamination; and the potential influences of remediation activities. Remaining uncertainties regarding specific sources of chromium at some locations, the direction and persistence of groundwater plumes and the specific location and extent of groundwater discharge into the Columbia River (Hope and Peterson 1996a, 1996b) should be assessed. Physical models and laboratory data should be examined in the field to verify the potential for exposure and to determine the probability of effect.

ACKNOWLEDGEMENTS

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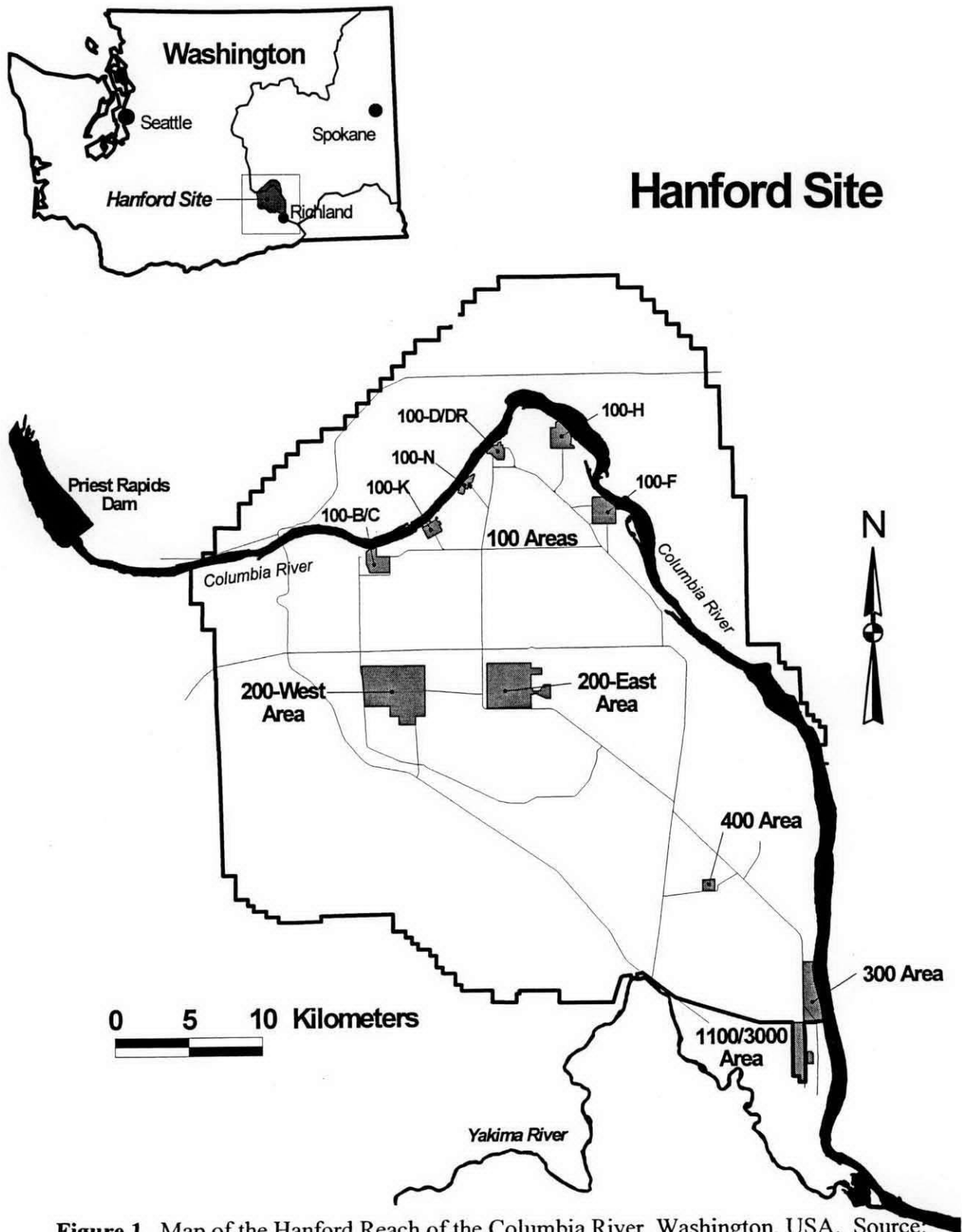


Figure 1. Map of the Hanford Reach of the Columbia River, Washington, USA. Source: Hanford Geographic Information System, Environmental Technologies Data Management, Bechtel Hanford, Inc.

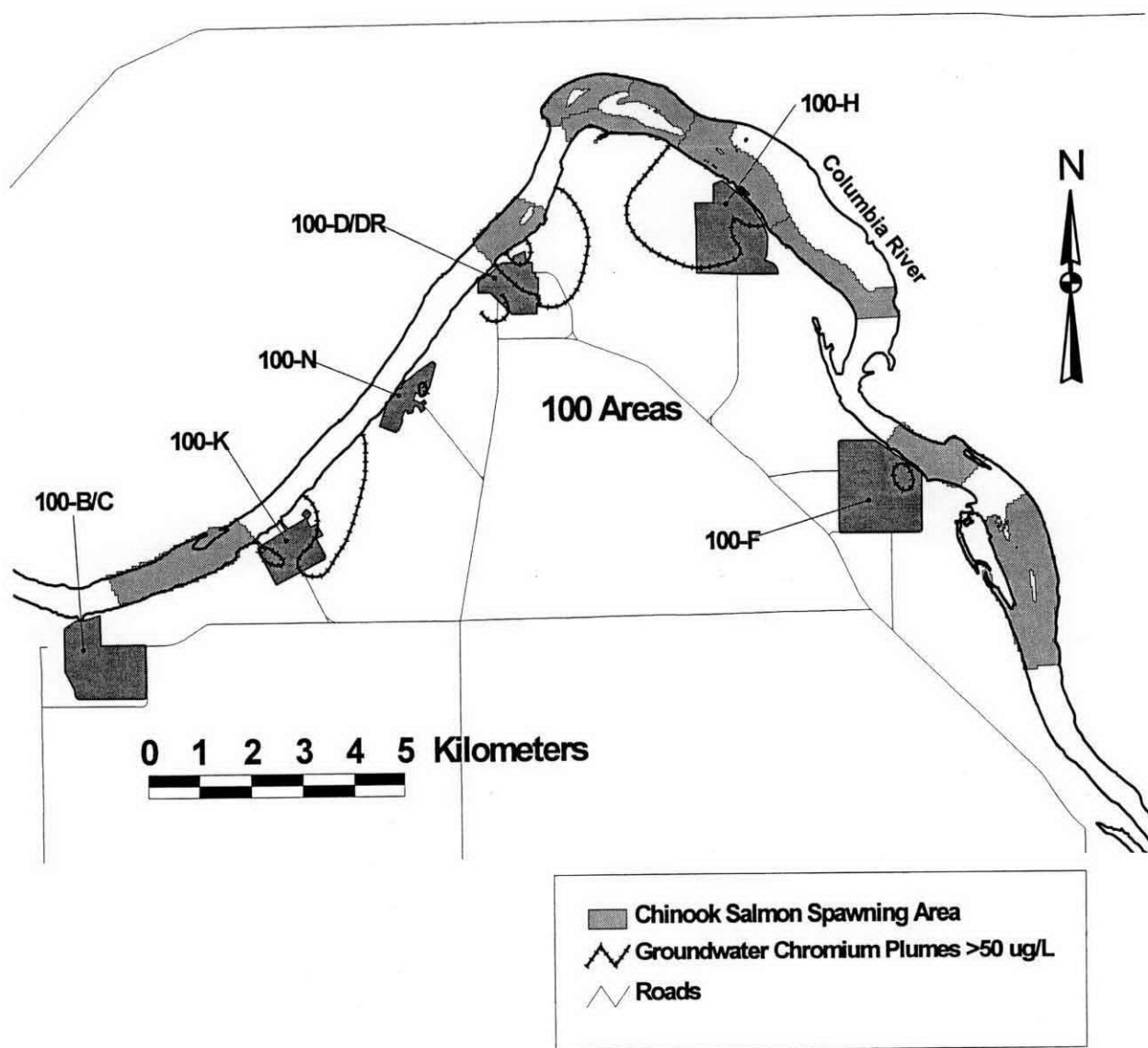


Figure 2. Map of the Hanford Reach of the Columbia River, Washington, USA that flows through the 100 Areas. Crosshatched areas within the river indicate locations of chinook salmon spawning redds. Groundwater plumes with levels of chromium exceeding 50 $\mu\text{g/L}$ are indicated by hatched contour lines. Source: Hanford Geographic Information System, Environmental Technologies Data Management, Bechtel Hanford, Inc.

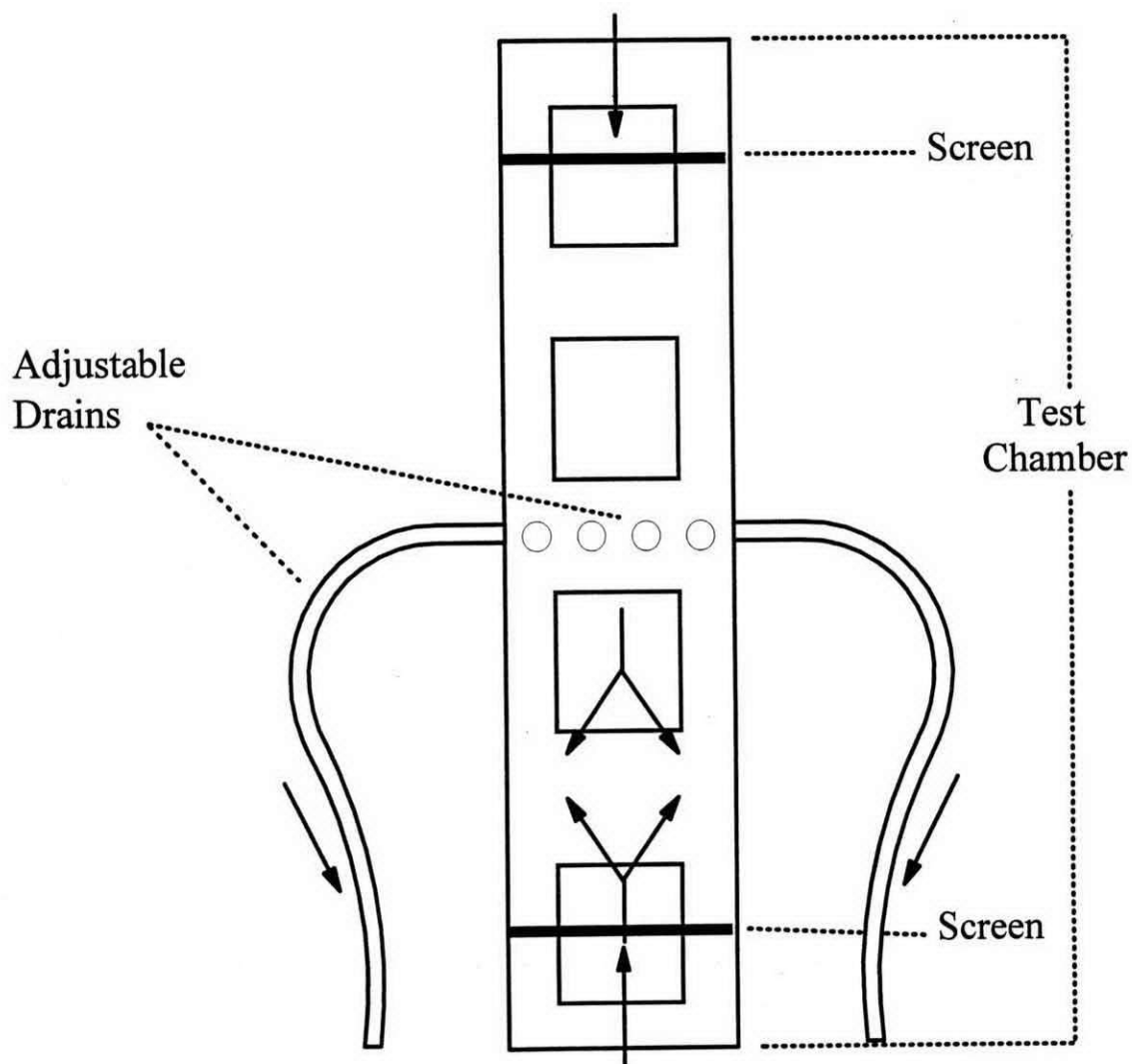


Figure 3. Illustration of an experimental chamber used in Experiments I and II. The chamber consists of a Plexiglas cylinder (11 cm diameter x 92 cm) with six centrally located drain holes. Openings are cut in the surface of the cylinder to allow the addition and removal of fish into the chamber and to allow the placement of screens at each end. Water (indicated by arrows) enters both ends of the chamber simultaneously and drains equally from six centrally located holes. The drains are adjustable to maintain the appropriate water level and contaminant gradient.

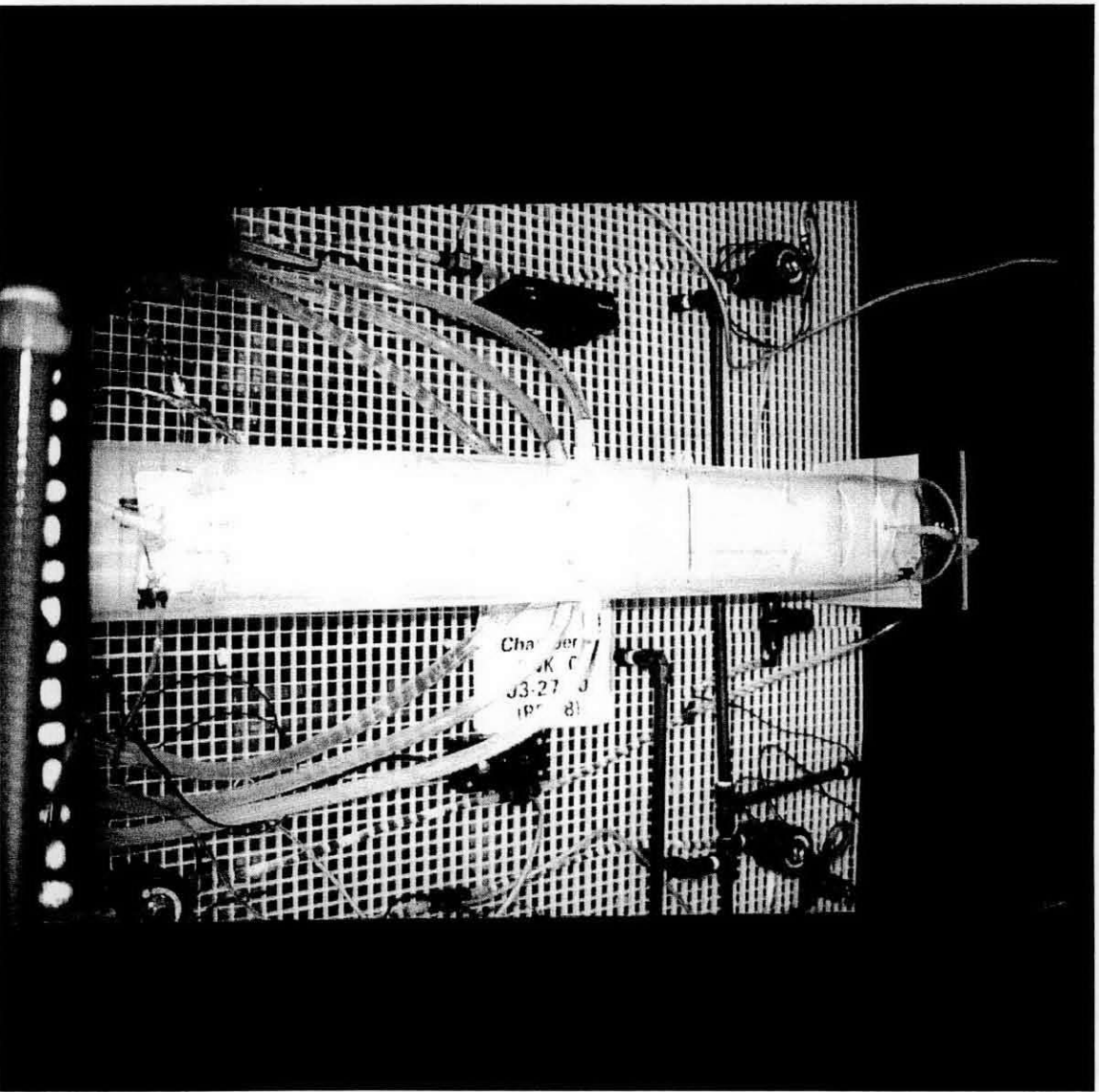


Figure 4. Photograph of an experimental chamber used in Experiments I and II.

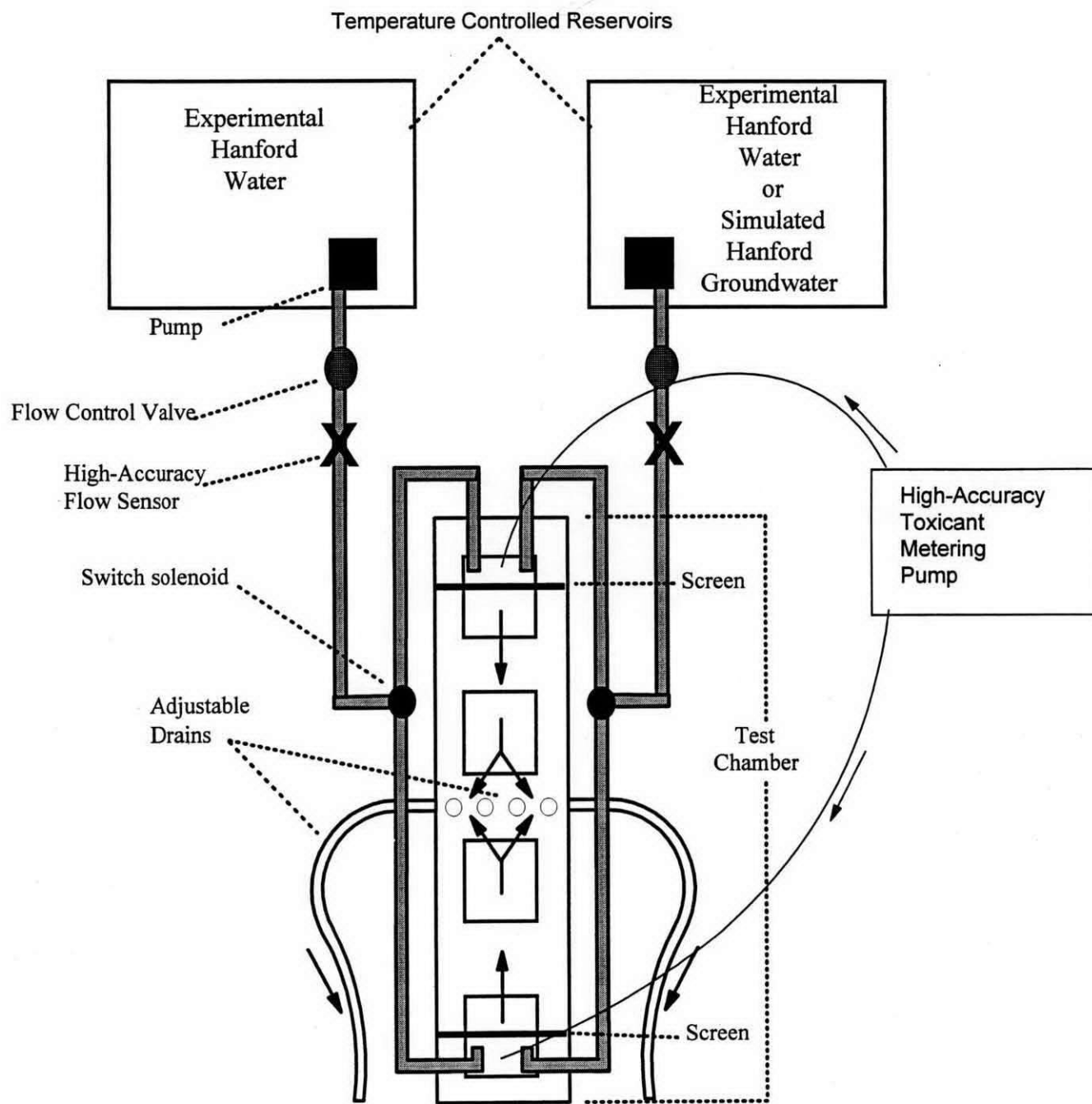


Figure 5. Illustration of the experimental apparatus (including an experimental chamber) used in Experiments I and II. Three identical chambers were used for each trial. The system delivered uncontaminated Hanford experimental water to one side of the chamber and the designated treatment combination to the other side. The side of the chamber receiving the chromium treatment was randomized between trials.

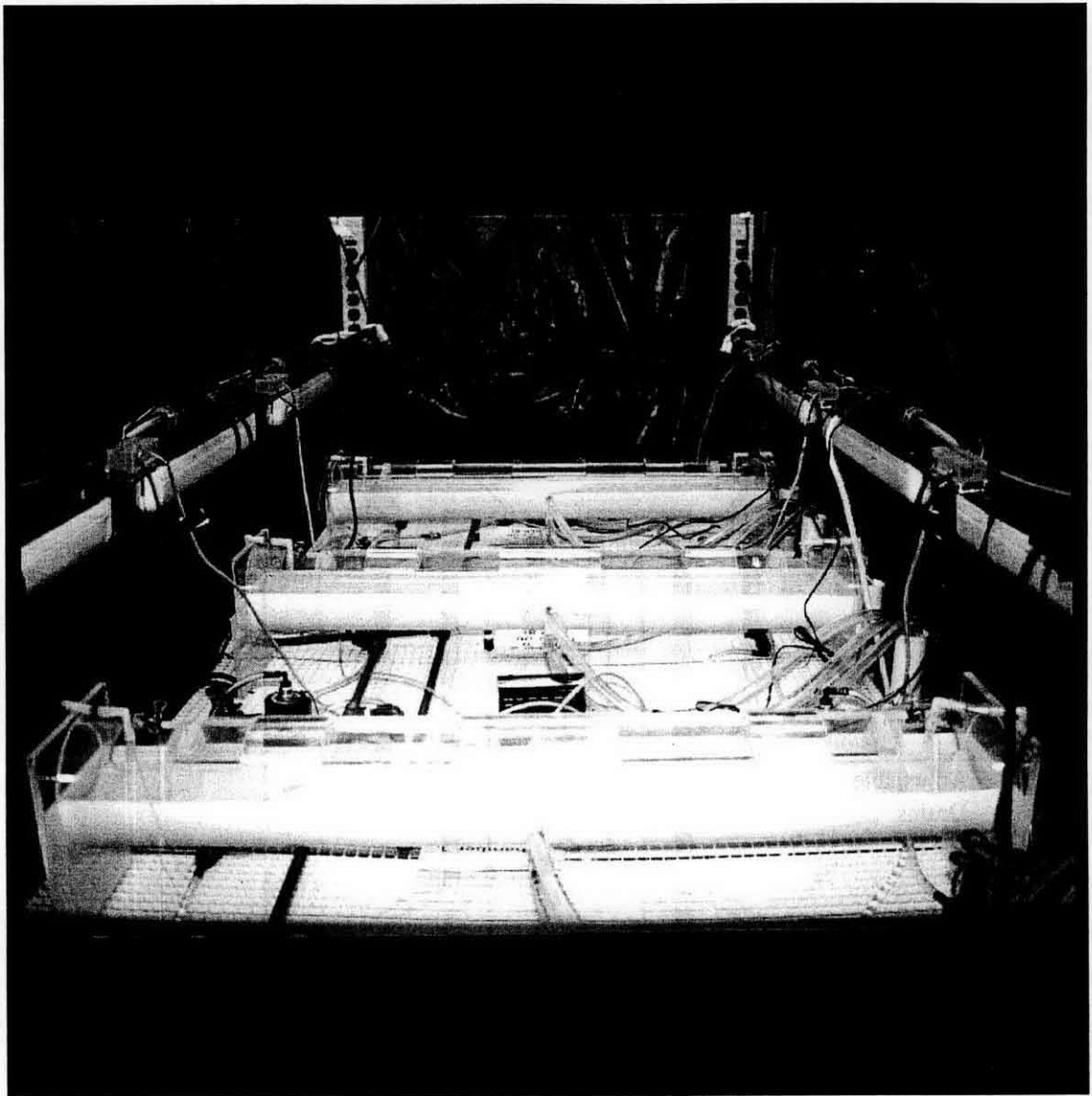


Figure 6. Photograph of the experimental apparatus used in Experiments I and II. A replicate consisted of three chambers with one fish in each. All three chambers in a replicate used the same treatment combination and were run simultaneously.

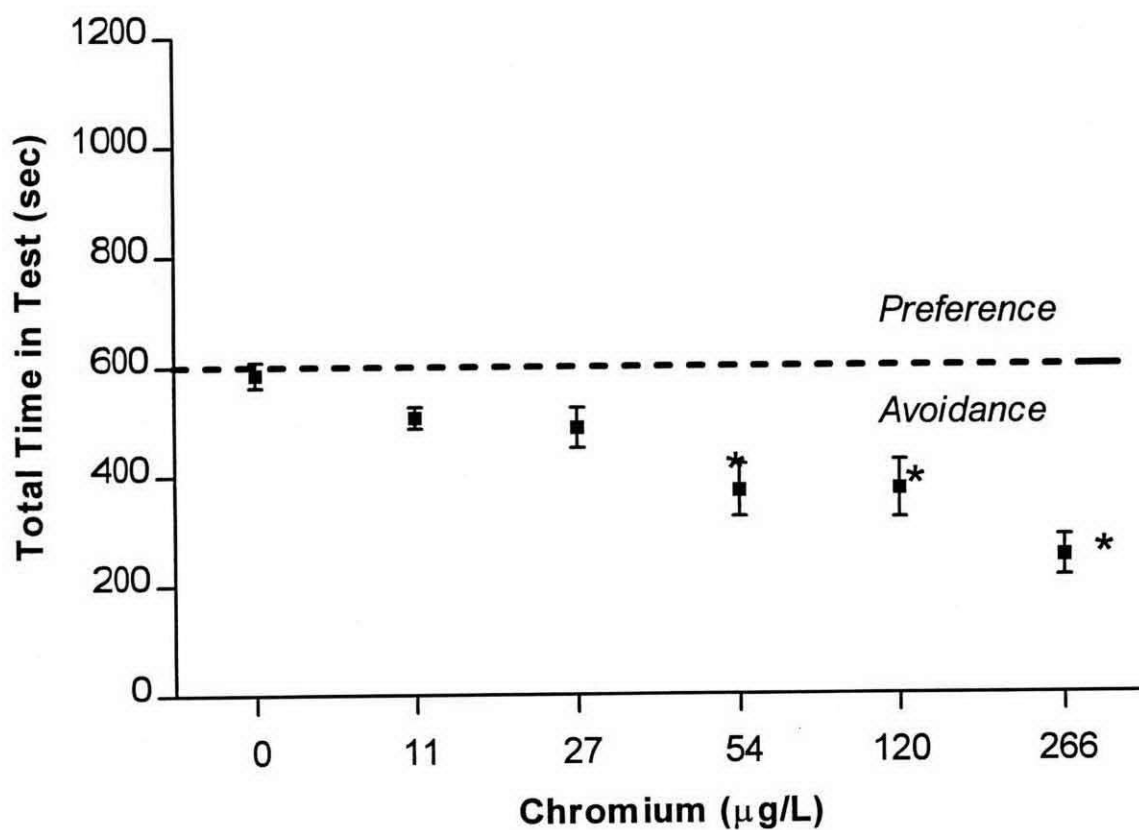


Figure 7. Mean (\pm standard error) of the total amount time spent in the treatment side of the test chamber during the observation period in Experiment I. The observation period was 1200 sec in duration. For all treatments $N=8$. The control condition was Hanford experimental water without chromium. The treatment condition was Hanford experimental water with or without chromium. Treatments noted with an asterisk are significantly different from the control condition ($P \leq 0.05$).

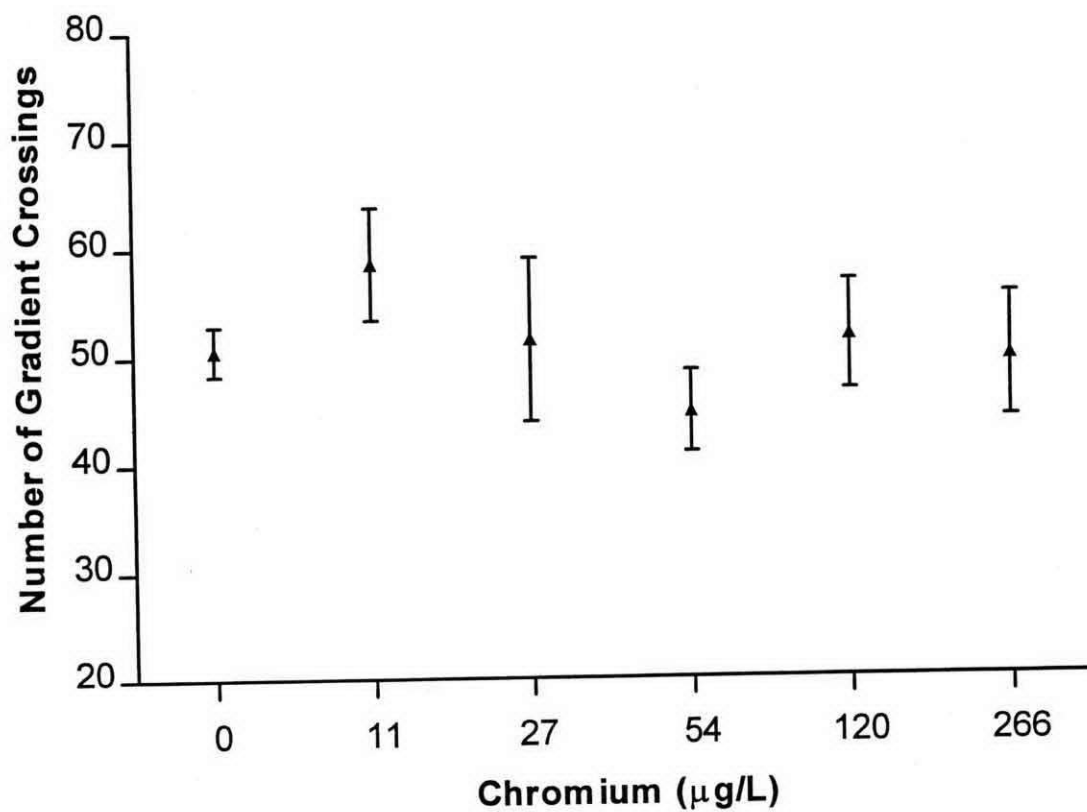


Figure 8. Mean (\pm standard error) frequency of gradient crossing into the treatment side of the chamber during the observation period in Experiment I. The observation period was 1200 sec in duration. For all treatments $N=8$. The control condition was Hanford experimental water without chromium. The treatment condition was Hanford experimental water with or without chromium. Treatments noted with an asterisk are significantly different from the control condition ($P \leq 0.05$).

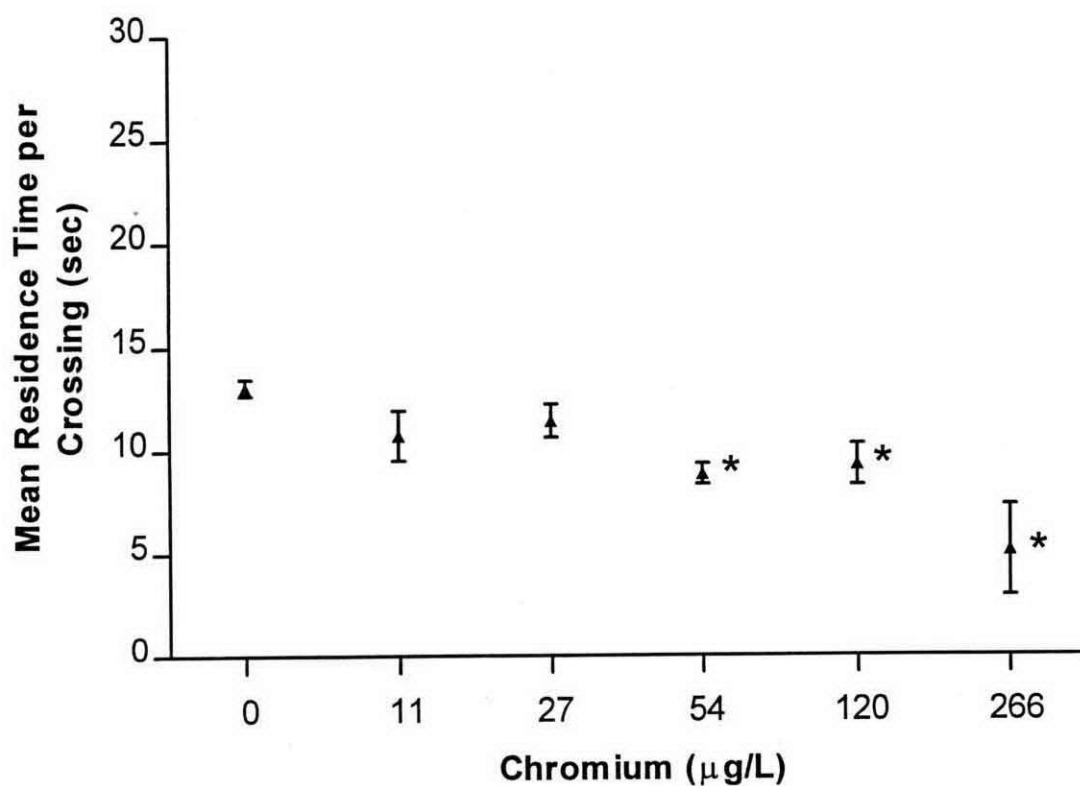


Figure 9. Mean (\pm standard error) residence time per gradient crossing into the treatment side (trip time) during the observation period in Experiment I. The observation period was 1200 sec in duration. For all treatments $N=8$. The control condition was Hanford experimental water without chromium. The treatment condition was Hanford experimental water with or without chromium. Treatments noted with an asterisk are significantly different from the control condition ($P \leq 0.05$).

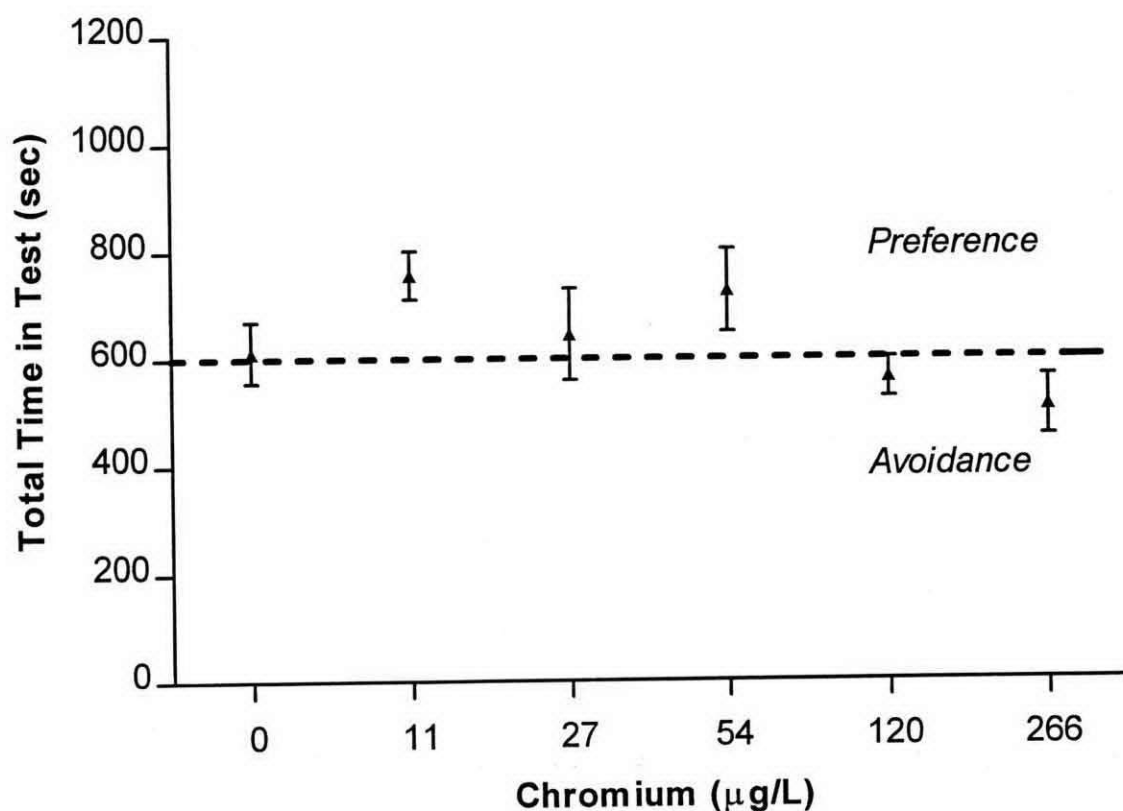


Figure 10. Mean (\pm standard error) of the total amount time spent in the treatment side of the test chamber during the observation period in Experiment II. The observation period was 1200 sec in duration. For all treatments $N=8$. The control condition was Hanford experimental water without chromium. The treatment condition was simulated Hanford groundwater with or without chromium. Treatments noted with an asterisk are significantly different from the control condition ($P \leq 0.05$).

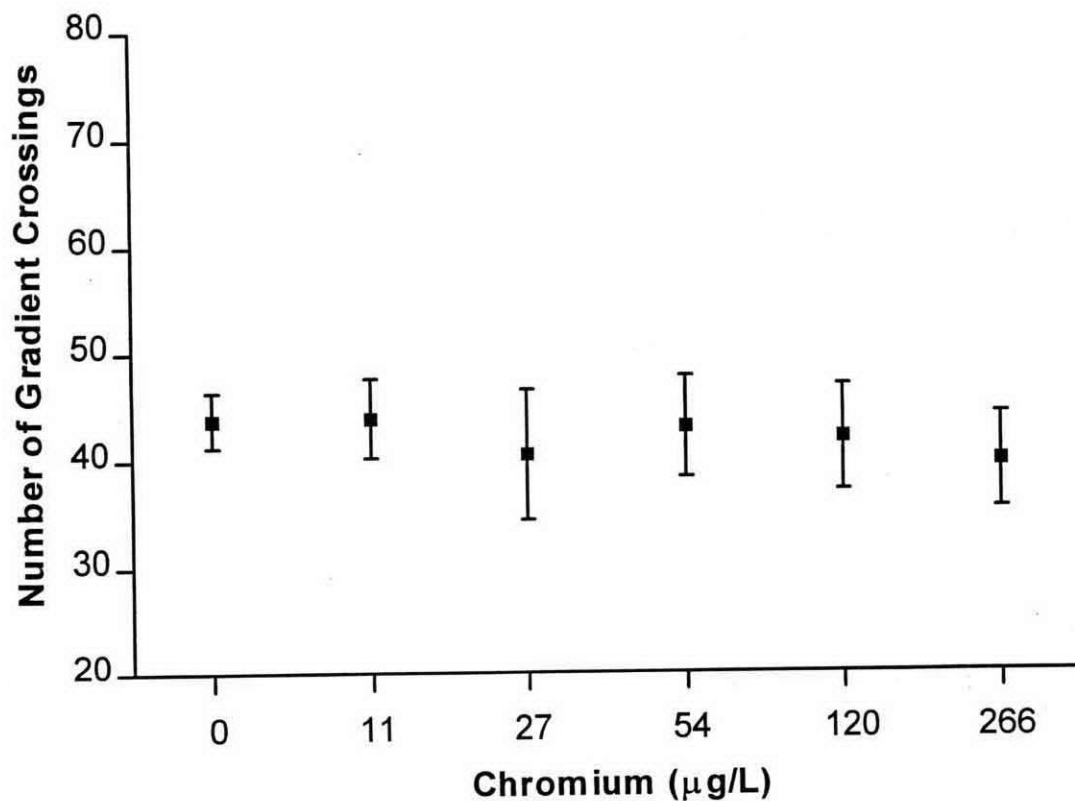


Figure 11. Mean (\pm standard error) frequency of gradient crossing into the treatment side of the chamber during the observation period in Experiment II. The observation period was 1200 sec in duration. For all treatments $N=8$. The control condition was Hanford experimental water without chromium. The treatment condition was simulated Hanford groundwater with or without chromium. Treatments noted with an asterisk are significantly different from the control condition ($P \leq 0.05$).

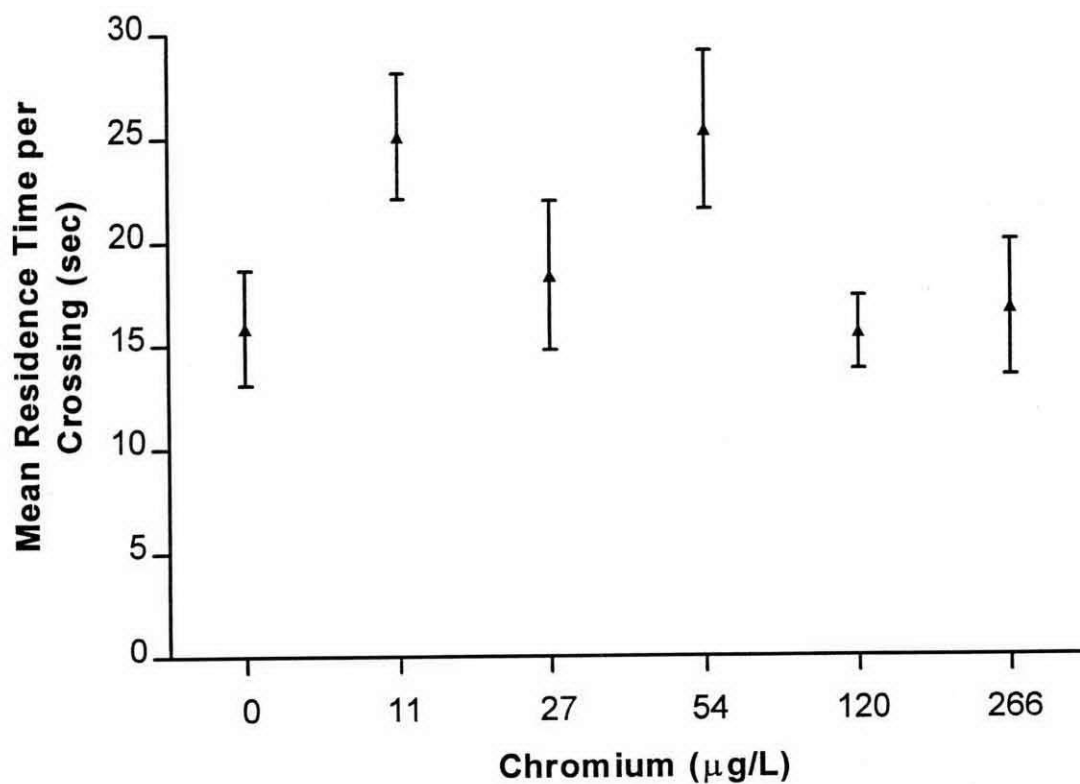


Figure 12. Mean (\pm standard error) residence time per gradient crossing into the treatment side (trip time) during the observation period in Experiment II. The observation period was 1200 sec in duration. For all treatments $N=8$. The control condition was Hanford experimental water without chromium. The treatment condition was simulated Hanford groundwater with or without chromium. Treatments noted with an asterisk are significantly different from the control condition ($P \leq 0.05$).

Table 1. Experimental matrix of treatment combinations used for behavioral experiments with chinook salmon parr.

Experiment I

Behavioral response of chinook salmon to chromium (VI) dissolved in Hanford Experimental Water¹

Treatment	Control side of chamber	Treatment side of chamber
0	80 mg/L Hardness + 0 µg/L Cr	80 mg/L Hardness + 0 µg/L Cr
11	80 mg/L Hardness + 0 µg/L Cr	80 mg/L Hardness + 11 µg/L Cr
27	80 mg/L Hardness + 0 µg/L Cr	80 mg/L Hardness + 27 µg/L Cr
54	80 mg/L Hardness + 0 µg/L Cr	80 mg/L Hardness + 54 µg/L Cr
120	80 mg/L Hardness + 0 µg/L Cr	80 mg/L Hardness + 120 µg/L Cr
266	80 mg/L Hardness + 0 µg/L Cr	80 mg/L Hardness + 266 µg/L Cr

Experiment II

Behavioral response of chinook salmon to chromium (VI) dissolved in Hanford Simulated Groundwater²

Treatment	Control side of chamber	Treatment side of chamber
0	80 mg/L Hardness + 0 µg/L Cr	200 mg/L Hardness + 0 µg/L Cr
11	80 mg/L Hardness + 0 µg/L Cr	200 mg/L Hardness + 11 µg/L Cr
27	80 mg/L Hardness + 0 µg/L Cr	200 mg/L Hardness + 27 µg/L Cr
54	80 mg/L Hardness + 0 µg/L Cr	200 mg/L Hardness + 54 µg/L Cr
120	80 mg/L Hardness + 0 µg/L Cr	200 mg/L Hardness + 120 µg/L Cr
266	80 mg/L Hardness + 0 µg/L Cr	200 mg/L Hardness + 266 µg/L Cr

¹ Experimental Water (80 mg/L hardness as CaCO₃)

² Simulated Groundwater (200 mg/L hardness as CaCO₃)

Table 2. Mean concentrations of measured total chromium in water sampled from the chromium-treated side of the experimental chamber during Experiment I. Chinook salmon parr were presented with a choice between a control condition (Hanford experimental water without chromium) and a test condition (Hanford experimental water with one of six concentrations of chromium).

Nominal Chromium ($\mu\text{g/L}$)	N	Mean Measured Total Chromium ($\mu\text{g/L}$)	Standard Deviation	Range
0	8	0.60	0.07	<0.57 – 0.75
11	8	11.0	0.86	9.52 – 12.4
27	8	26.7	0.99	25.5 – 28.2
54	8	52.8	1.6	50.2 – 54.7
120	8	120.2	10.1	114 – 145
266	8	256.5	8.1	247 - 274

Table 3. Mean concentrations of measured total chromium in water sampled from the chromium-treated side of the experimental chamber during Experiment II. Chinook salmon parr were presented with a choice between a control condition (Hanford experimental water without chromium) and a test condition (Hanford simulated groundwater water with one of six concentrations of chromium).

Nominal Chromium (µg/L)	N	Mean Measured Total Chromium (µg/L)	Standard Deviation	Range
0	8	0.67	0.45	<0.29 – 1.6
11	8	11.4	0.87	10.3 – 12.6
27	8	26.7	0.85	24.8 – 27.4
54	8	53.6	1.4	51.4 – 55.3
120	8	117.2	3.0	113 – 123
266	8	256.1	3.9	251 -261

Table 4. Mean weights and lengths of chinook salmon parr tested during Experiment I. Standard Errors of the Means (SEM) are in parentheses. Lengths and weights were statistically similar among all treatments.

Nominal Chromium ($\mu\text{g/L}$)	N	Weight (g)	Length (cm)
0	8	1.176 (0.035)	5.46 (0.08)
11	8	1.244 (0.058)	5.57 (0.07)
27	8	1.298 (0.036)	5.66 (0.05)
54	8	1.307 (0.076)	5.62 (0.09)
120	8	1.261 (0.064)	5.60 (0.07)
266	8	1.339 (0.055)	5.68 (0.07)

Table 5. Mean weights and lengths of chinook salmon parr tested during Experiment II. Standard Errors of the Means (SEM) are in parentheses. Lengths and weights were statistically similar among all treatments.

Nominal Chromium ($\mu\text{g/L}$)	N	Weight (g)	Length (cm)
0	8	1.311 (0.065)	5.52 (0.08)
11	8	1.277 (0.088)	5.48 (0.12)
27	8	1.242 (0.076)	5.43 (0.10)
54	8	1.335 (0.089)	5.52 (0.10)
120	8	1.400 (0.081)	5.64 (0.09)
266	8	1.356 (0.052)	5.55 (0.06)

Table 6. Behavioral response of chinook salmon parr for Experiment I as measured by the total time spent in the contaminated portion of the experimental chamber, percent of the total test period spent in the contaminated side, the mean residence time of each excursion into the contaminated side, and the mean number of times the test fish crossed the gradient from the control to the treatment side. The duration of the test period was 1200 sec. Standard Errors of the Mean (SEM) are in parenthesis.

Nominal Chromium ($\mu\text{g/L}$)	N	Time (sec) spent in contaminant	Percent of time spent in contaminant	Mean residence time in contaminant	Mean number of gradient crossings
0	8	586.2 (23.5)	48.85 (2.0)	13.08 (0.4)	50.6 (2.3)
11	8	507.1 (19.9)	42.26 (1.7)	10.77 (1.2)	58.6 (5.2)
27	8	488.2 (37.2)	40.68 (3.1)	11.46 (0.8)	51.5 (7.6)
54	8	372.6 ^a (48.3)	31.05 ^a (4.0)	8.88 ^a (0.5)	44.8 (3.8)
120	8	374.7 ^a (53.0)	31.22 ^a (4.4)	9.33 ^a (1.0)	51.8 (5.1)
266	8	250.8 ^a (36.9)	20.90 ^a (3.1)	5.17 ^a (2.2)	49.8 (5.7)

^a indicates that the treatment was significantly different from the control condition ($P \leq 0.05$).

Table 7. Behavioral response of chinook salmon parr for Experiment II as measured by the total time spent in the contaminated portion the experimental chamber, percent of the total test period spent in the contaminated side, the mean residence time of each excursion into the contaminated side, and the mean number of times the test fish crossed the gradient from the control to the treatment side. The duration of the test period was 1200 sec. Standard Errors of the Mean (SEM) are in parenthesis.

Nominal Chromium ($\mu\text{g/L}$)	N	Time spent in contaminant (sec)	Percent of time spent in contaminant	Mean residence time in contaminant (sec)	Mean number of gradient crossings
0	8	612.7 (57.6)	51.1 (4.8)	15.9 (2.8)	43.8 (2.6)
11	8	757.7 (44.9)	63.1 (3.7)	25.1 (3.0)	43.9 (3.7)
27	8	645.3 (86.0)	53.8 (7.2)	18.4 (3.6)	40.5 (6.1)
54	8	726.8 (77.5)	60.6 (6.5)	25.4 (3.8)	43.1 (4.7)
120	8	563.1 (37.3)	46.9 (3.1)	15.6 (1.8)	42.0 (4.9)
266	8	509.3 (56.3)	42.4 (4.7)	16.8 (3.3)	39.8 (4.4)

^a indicates that the treatment was significantly different from the control condition ($P \leq 0.05$).

Table 8. Comparison of behavioral responses to chromium (VI) determined in the present study with results reported in previous studies.

Study	Species	Test Conditions	Culture conditions	Behavioral response	Reported effective concentration
<i>Present study</i>					
Experiment I	chinook salmon (<i>Oncorhynchus tshawytscha</i>)	Hardness 80 mg/L Alkalinity 72 mg/L pH 8.1	Same as test conditions	No response	≤27 µg/L
				Avoidance	≥ 54 µg/L
Experiment II	chinook salmon	Hardness 200 mg/L Alkalinity 178 mg/L pH 8.3	Hardness 80 mg/L Alkalinity 72 mg/L pH 8.1	No response	0 – 266 µg/L
<i>Previous studies</i>					
Anestis and Neufeld 1986	rainbow trout (<i>Oncorhynchus mykiss</i>)	Hardness 100 mg/L Alkalinity 50 mg/L pH 7.2	Same as test conditions	Avoidance	> 28 µg/L
Hartwell et al. 1987	golden shiner (<i>Notemigonus crysoleucas</i>)	Hardness 72 mg/L Alkalinity 42 mg/L pH 7.5	Same as test conditions	Avoidance	≥ 73 µg/L

REFERENCES

- Anestis, I. and R. Neufeld. 1986. Avoidance-preference reactions of rainbow trout (*salmo gairdneri*) after prolonged exposure to chromium (VI). *Water Resources* 20: 1223-1241.
- ASTM (American Society for Testing and Materials). 2000a. Annual Book of ASTM Standards. Volume 11.05. E1241-98. Standard Guide for Conducting Early Life-Stage Toxicity Tests with Fishes. Philadelphia, Pennsylvania. 29 pp.
- ASTM (American Society for Testing and Materials). 2000b. Annual Book of ASTM Standards. Volume 11.05. E1850-97. Standard Guide for Selection of Resident Species as Test Organisms for Aquatic and Sediment Toxicity Tests. Philadelphia, Pennsylvania. 13 pp.
- Atchison, G. J., M.G. Henry, and M.B. Sandheinrich. 1987. Effects of metals on fish behavior: a review. *Environmental Biology of Fishes* 18: 11-25.
- Becker, C.D. 1973. Food and growth parameters of juvenile chinook salmon, *Oncorhynchus tshawytscha*, from the central Columbia River. U.S. National Marine Fisheries Service Fishery Bulletin 71:387-400.
- Becker, C.D. 1985. Anadromous salmonids of the Hanford Reach, Columbia River: 1984 status. Battelle, Pacific Northwest Laboratory, PNL-5371 Richland, Washington.
- Beitinger, T. L. 1990. Behavioral reactions for the assessment of stress in fishes. *Journal of Great Lakes Research* 16: 495-528.
- Benoit, D.A. 1976. Toxic effects of hexavalent chromium on brook trout (*Salvelinus fontinalis*) and rainbow trout (*Salmo gairdneri*). *Water Research* 10: 497-500.
- Buhl, K.J. and S.J. Hamilton. 1991. Relative sensitivity of early life stages of arctic grayling, coho salmon, and rainbow trout to nine inorganics. *Ecotoxicology and Environmental Safety* 22:184-197.
- Black, J.A., R.F. Roberts, D.M. Johnson, D.D. Minicucci, K.H. Mancy and H.E. Allen. 1973. The significance of physiochemical variables in aquatic bioassays of heavy metals. In G.E. Glass, ed., *Bioassay Techniques and Environmental Chemistry*. Ann Arbor Science Publishers, Ann Arbor, MI. 259-275.

- Dauble, D.D. and D.G. Watson. 1997. Status of fall chinook salmon populations in the mid-Columbia River, 1948-1992. *North American Journal of Fisheries Management* 17:283-300.
- Dauble, D.D. and D.G. Watson. 1990. Spawning and abundance of fall chinook salmon (*Oncorhynchus tshawytscha*) in the Hanford Reach of the Columbia River, 1948-1988. PNL-7289, Pacific Northwest Laboratory, Richland, Washington.
- Dauble, D.D., T.L. Page, and R.W. Hanf, Jr. 1989. Spatial distribution of juvenile salmonids in the Hanford Reach, Columbia River. *U.S. National Marine Fisheries Service Fishery Bulletin* 87:775-790.
- DeLonay, A.J., E.E. Little, J.J. Lipton, D.F. Woodward, and J. Hansen. 1996. Behavioral avoidance as evidence of injury to fishery resources: Applications to natural resource damage assessments. In: T.W. LaPoint, F.T. Price, and E.E. Little, eds., *Environmental Toxicology and Risk Assessment: Fourth Volume*, ASTM STP 1262, American Society for Testing and Materials, Philadelphia. pp. 268-280.
- Farag, A.M., A.J. DeLonay, W.G. Brumbaugh, E.E. Little, L. Cleveland and D.F. Woodward. 2000. The potential for chromium to adversely affect chinook salmon (*Oncorhynchus tshawytscha*) in the Hanford Reach of the Columbia River, Washington, USA. U.S. Geological Survey Final Report to the U.S. Fish and Wildlife Service. Columbia Environmental Research Center, Columbia, Missouri. 53 p.
- Geckler, J. R., W.B. Horning, T.M. Neiheisel, Q.H. Pickering, E.L. Robinson and C.E. Stephan. 1976. Validity of laboratory tests for predicting copper toxicity in streams. U.S. Environmental Protection Agency EPA-600/3-76-116. Environmental Research Laboratory, Duluth, Minnesota.
- Geist, D.R. 2000. Hyporheic discharge of river water into fall chinook salmon (*Oncorhynchus tshawytscha*) spawning areas in the Hanford Reach, Columbia River. *Can J fish Aquat Sci* 57:1647-1656.
- Geist, D.R., J. Jones, C.J. Murray and D.D. Dauble. 2000. Suitability criteria analyzed at the spatial scale of redd clusters improved estimates of fall chinook salmon (*Oncorhynchus tshawytscha*) spawning habitat use in the Hanford Reach, Columbia River. *Can J fish Aquat Sci* 57:1636-1646.

- Geist, D.R. 1997. Characterization of spawning habitat within a high and a low density of fall chinook salmon spawning area in the Hanford Reach, Columbia River. Unpublished report by Battelle Pacific Northwest National Laboratory.
- Geist, D.R. 1995. The Hanford Reach: what do we stand to lose? *Illahee* 2:130-141.
- Geist, D.R., T.M. Poston and D.D. Dauble. 1994. Assessment of potential impacts of major groundwater contaminants to fall chinook salmon (*Oncorhynchus tshawytscha*) in the Hanford Reach, Columbia River. Pacific Northwest Laboratory, PNL-9990, Richland, Washington.
- Hamilton, S.J. and K.J. Buhl. 1990. Safety assessment of selected inorganic elements to fry of chinook salmon (*Oncorhynchus tshawytscha*). *Ecotoxicology and Environmental Safety* 20: 307-324.
- Hansen, J.A., D.F. Woodward, E.E. Little, A.J. DeLonay, and H.L. Bergman. 1999. Behavioral avoidance: A possible mechanism for explaining abundance and distribution of trout species in a metals impacted river. *Environmental Toxicology and Chemistry* 18:313- 317.
- Hansen, J.A., J.C.A. Marr, J. Lipton, D. Cacela, and H.L. Bergman. 1999a. Differences in neurobehavioral responses of chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*O. mykiss*) exposed to copper and cobalt: Behavioral avoidance. *Environmental Toxicology and Chemistry* 18(9):1972-1978.
- Hansen, J.A., J.D. Rose, R.A. Jenkins, K.G. Gerow, and H.L. Bergman. 1999b. Chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*Oncorhynchus mykiss*) exposed to copper: Neurophysiological and histological effects on the olfactory system. *Environmental Toxicology and Chemistry* 18(9):1979-1991.
- Hartwell, S.I., J.H. Jin, D.S. Cherry and J. Cairns, Jr. 1989. Toxicity versus avoidance response of golden shiner, *Notemigonus crysoleucas*, to five metals. *Journal of Fish Biology* 35:447-456.
- Hartwell, S.I., D.S. Cherry and J. Cairns, Jr. 1987. Field validation of avoidance of elevated metals by fathead minnows (*Pimephales promelas*) following in situ acclimation. *Environmental Toxicology and Chemistry* 6: 189-200.

- Hope, S.J., and R.E. Peterson. 1996a. Chromium in river substrate pore water and adjacent groundwater: 100-D/DR Area, Hanford Site, Washington. Betchel Hanford, Inc, Richland Washington. BHI-00778 Rev. 0.
- Hope, S.J., and R.E. Peterson. 1996b. Pore water chromium concentrations at 100-H reactor area adjacent to fall chinook salmon spawning habitat of the Hanford Reach, Columbia River. Betchel Hanford, Inc, Richland Washington. BHI-00345 Rev. 0.
- Lipton, J.J., E.E. Little, J.C.A. Marr, and A.J. DeLonay. 1996. Use of behavioral avoidance testing in natural resource damage assessment. In: D.A. Bengston and D. Henshel, eds., *Environmental Toxicology and Risk Assessment: Fifth Volume*, ASTM STP 1306, American Society for Testing and Materials, Philadelphia. pp. 310-322.
- Little, E.E., B.A. Flerov and N.N. Ruzhinskaya. 1985. Behavioral approaches in aquatic toxicity investigations: a review. In P.M. Merhle, Jr., R.H. Gray and R.L. Kendall (eds.). *Toxic substances in the aquatic environment: an international aspect*. American Fisheries Society, Bethesda, Maryland.
- Little, E. E., J.J. Fairchild and A.J. DeLonay. 1993. Behavioral methods for assessing impacts of contaminants on early life stage fishes. *Water Quality and the Early Life Stages of Fishes*, American Fisheries Society Symposium 14: 67-76.
- Mayer, F.L., Jr. and M.R. Ellersieck. 1986. *Manual of Acute Toxicity: Interpretation and Database for 410 chemicals and 66 Species of Freshwater Animals*. U.S. Fish and Wildlife Service, Resource Publication 160. 579 pp.
- Marr, J., J. Lipton, and D. Cacela. 1995. *Fisheries Toxicity Injury Studies Blackbird Mine Site, Idaho*. National Oceanic and Atmospheric Administration. Report No. 50-DGNC-1-00007, Washington, D.C.
- Olson, P.A. and R.F. Foster. 1956. Effect of chronic exposure to sodium dichromate on young chinook salmon and rainbow trout. *In Hanford Biological Research Annual Report, 1955*. Atomic Energy Commission, HW-41500, Richland, Washington.
- Patton, G.W., D.D. Dauble, M.A. Chamness, C.S. Abernathy and C.A. McKinstry. 2001. Chromium toxicity test for fall Chinook salmon (*Oncorhynchus tshawytscha*) using Hanford site groundwater; Onsite Early life stage toxicity evaluation. Pacific Northwest National Laboratory, Richland, Washington.

- Peterson, R.E., R.F. Raidle, and C.W. Denslow. 1996. Conceptual Site Models for Groundwater Contamination at 100-BC-5, 100-KR-4, 100-HR-3, and 100-FR-3 Operable Units. Bechtel Hanford, Inc., BHI-00917, Richland, Washington.
- Saunders, R.L. and J.B. Sprague. 1967. Effects of copper-zinc mining pollution on a spawning migration of Atlantic salmon. *Water Res.* 1: 419-432.
- Sprague, J. B., P.F. Elson, and R.L. Saunders. 1965. Sublethal copper-zinc pollution in a salmon river--A field and laboratory study. *International Journal of Air and Water Pollution* 9: 531-543.
- Sprague, J.B. 1968. Avoidance of copper-zinc solutions by young salmon in the laboratory. *J. Water Pollut. Control Fed.* 36: 990-1004.
- Swan, G.A., E.M. Dawley, R.D. Ledgerwood, W.T. Norman, W.F. Cobb, and D.T. Hartman. 1988. Distribution and relative abundance of deep-water redds for spawning fall chinook salmon at selected study sites in the Hanford Reach of the Columbia River. Report E86-87-3082, U.S. Army Corps of Engineers and U.S. National Marine Fisheries Service.
- USEPA (United States Environmental Protection Agency). 1986. Quality Criteria for Water 1986. EPA Report 440/5-86-001, Office of Water Regulations and Standards, Washington, D.C.
- Wiggins, W.D., G.P. Ruppert, R.R. Smith, L.L. Reed, L.E. Hubbard, and M.L. Courts. 1997. Water Resources Data Washington Water Year 1996. U.S. Geological Survey Water Data Report WA-96-1, Tacoma, Washington.
- Woodward, D.F., J.A. Hansen, H.L. Bergman, E.E. Little, and A.J. DeLonay. 1995. Brown trout avoidance of metals in water characteristic of the Clark Fork River, Montana. *Canadian Journal of Fisheries and Aquatic Sciences* 52:2031-2037.